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In re Application of:

Yoseph SHAALTIEL

Serial No.: 10/784,295

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For: CELL/TISSUE CULTURING DEVICE,
SYSTEM AND METHOD

Group Art Unit: 1744

Attorney
Docket: 27557

Examiner:

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

CLAIM OF PRIORITY RIGHTS

Sir:

Applicant hereby perfects the claimed priority date of Israel Patent Application No. 119310 filed 26 September 1996, and encloses herewith a certified copy of that Israel Patent Application to support the claim for its priority date.

Respectfully submitted,

Martin D. Nozickman

Martin Moynihan
Registration Number 40,338

Date: 10 November 2004



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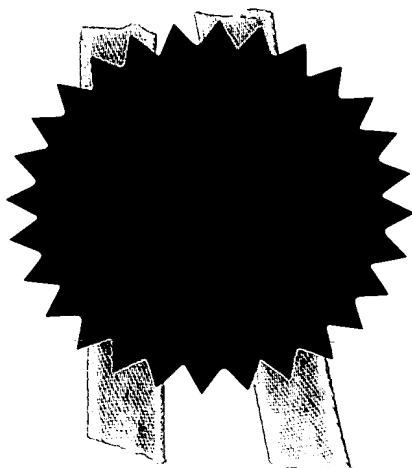
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מספר: Number	119310
תאריך: Date	26-09-1996
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Application for Patent

וני, (שם, המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
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התקן ושיטה לגידול תאים/רקמות


(English)

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CELL/TISSUE CULTURING DEVICE AND METHOD

hereby apply for a patent to be granted to me in respect thereof.

בבקש בזאת כי ינתן עליה פטנט

• בקשת חלוקה - Application of Division		• בקשת פטנט מוסף - Application for Patent Addition		דרישת דין קדימה Priority Claim		
מבקשת פטנט from Application		לבקשה/לפטנט to Patent/Appl.		מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
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המען למסירת מסמכים בישראל Address for Service in Israel A.E. MULFORD P.O. Box 544 JERUSALEM 91004 א.י. מלפורד ת.ד. 544 ירושלים 91004						
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התקן ושיטה לגידול תאים/רקמות

CELL/TISSUE CULTURING DEVICE AND METHOD

FIELD OF INVENTION

The present invention relates to devices for culturing and harvesting cells and/or tissues, bioreactors and fermentors. In particular this invention relates to such devices which are disposable but which nevertheless may be used a plurality of times prior to disposal of same.

INTRODUCTION

Cell and tissue culture techniques have been available for many years and are well known in the art. The prospect of using such culturing techniques economically is for the extraction of secondary metabolites, such as pharmaceutically active compounds, various substances to be used in cosmetics, food additives and natural pesticides, from a harvest of the cultured cells or tissues. While potentially lucrative, this prospect has nevertheless not crystallised. Prior art technology for the production of cell and/or tissue culture in commercial quantities, to be used for the extraction of secondary metabolites, is based on glass bioreactors and stainless steel bioreactors. These bioreactors represent expensive capital items, and are in general each provided with substantially the peak volume capacity that is required at the time. The alternative, namely to provide a number of smaller bioreactors whose total volume capacity matches requirements, while offering a degree of flexibility for increasing or reducing overall capacity, is nevertheless much more expensive than the provision of a single larger bioreactor. Furthermore, running costs associated with glass and stainless steel bioreactors are also high, due to low yields coupled to the need for sterilising the bioreactors after every culturing cycle. Consequently, the products extracted from cells or tissues grown in such bioreactors are expensive, and cannot at present compete commercially with comparable products produced with

alternative techniques. In fact, only one Japanese company is known to use the aforementioned cell/tissue culture technique commercially, using stainless steel bioreactors. This company produces Shikonin, a compound which is used exclusively in Japan.

Disposable bioreactors may also be used for culturing cells and/or tissues and may thus provide an alternative solution to glass and stainless steel bioreactors. One such known disposable bioreactor is produced by Osmotec, Israel, and comprises a conical bag having an inlet through which culture medium, air, inoculant and other optional additives may be introduced. However, these bags are used mainly in laboratory research and their volume is determined by the overall volume capacity requirements, as for the stainless steel bioreactors. Mixing of the medium is performed by very small air bubbles which in many cases results in damage to cell walls, particularly in the case of plant cell cultures. In particular, these bags are for single-use only, and are therefore not directed towards an economical solution to the question of providing commercial quantities of secondary metabolites to be extracted from the culture, as discussed above.

An aim of the present invention is to provide a device, and associated method, for culturing and harvesting cells and/or tissue which does not have the foregoing disadvantages, and which furthermore is economical and simple to produce and to use.

Another aim of the present invention is to provide such a device which is disposable, but nevertheless may be used a plurality of times for cyclically culturing and harvesting desired cells and/or tissues.

Another aim of the present invention is to provide such a device wherein inoculant is only required to be provided for the first culturing cycle, while inoculant for subsequent cycles is provided by a portion of the culture broth which remains in the device after harvesting same in a preceding cycle.

Another aim of the present invention is to provide a battery of such devices for industrial scale production of cells and/or tissues.

SUMMARY OF THE INVENTION

A multiple-use disposable device, and corresponding method, for culturing and harvesting cells and/or tissue in cycles comprising a sterilisable transparent and/or translucent reservoir having a top end and a bottom end, which reservoir may be filled with a suitable sterile biological cell and/or tissue culture medium and/or sterile inoculant and/or sterile air and/or other sterile required additives, said reservoir comprising: air inlet means for introducing sterile air in the form of bubbles into said culture medium through a first inlet opening connectable to a suitable air supply; gas outlet means for removing excess air and/or waste gases from said reservoir connectable to a suitable exhaust means, said exhaust means comprising means for substantially preventing introduction of contaminants into said reservoir via said gas outlet means; additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said reservoir, said additive inlet means comprising means for substantially preventing introduction of contaminants into said reservoir via said additive inlet means; and harvesting means for harvesting a desired first portion of the said medium containing cells and/or tissue, wherein a

remaining second portion of said medium containing cells and/or tissue serve as inoculant for a next culture and harvest cycle wherein said culture medium and/or said required additives provided, said harvest means comprising means for substantially preventing introduction of contaminants into said reservoir via said harvesting means; wherein said device may be utilised a plurality of times for culturing and harvesting said cells and/or tissue in cycles, wherein inoculant is externally provided for a first cycle thereof, and wherein for each subsequent cycle, said second portion of medium with cell/tissue remaining after a preceding harvest serves as inoculant for the next cycle, and wherein medium and air and any other required additives are provided in suitable quantities during each cycle to enable culture of said cells and/or tissue from said inoculant, and wherein said device may be disposed of when contaminated.

DESCRIPTION OF FIGURES

Figure 1 illustrates the main components of a preferred embodiment of the present invention.

Figure 2 illustrates the main components of an embodiment of the battery of the present invention.

DESCRIPTION

The present invention relates to a multiple-use disposable device for culturing and harvesting cells and/or tissue in cycles comprising a sterilisable transparent and/or translucent reservoir having a top end and a bottom end, which reservoir may be filled with a suitable sterile biological cell and/or tissue culture medium and/or sterile inoculant and/or sterile air and/or other sterile required additives, said reservoir comprising:-

- (i) air inlet means for introducing sterile air in the form of bubbles into said culture medium through a first inlet opening connectable to a suitable air supply;
- (ii) gas outlet means for removing excess air and/or waste gases from said reservoir connectable to a suitable exhaust means, said exhaust means comprising means for substantially preventing introduction of contaminants into said reservoir via said gas outlet means;
- (iii) additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said reservoir, said additive inlet means comprising means for substantially preventing introduction of contaminants into said reservoir via said additive inlet means; and
- (iv) harvesting means for harvesting a desired first portion of the said medium containing cells and/or tissue, wherein a remaining second portion of said medium containing cells and/or tissue serve as inoculant for a next culture and harvest cycle wherein said culture medium and/or said required additives provided, said harvest means comprising means for substantially preventing introduction of contaminants into said reservoir via said harvesting means;

wherein said device may be utilised a plurality of times for culturing and harvesting said cells and/or tissue in cycles, wherein inoculant is externally provided for a first

cycle thereof, and wherein for each subsequent cycle, said second portion of medium with cell/tissue remaining after a preceding harvest serves as inoculant for the next cycle, and wherein medium and air and any other required additives are provided in suitable quantities during each cycle to enable culture of said cells and/or tissue from said inoculant, and wherein said device may be disposed of when contaminated.

Thus, with reference to Figure 1, a preferred embodiment of the device, generally designated (10), comprises a transparent and/or translucent reservoir (20), having a top end (26) and a bottom end (28). In the preferred embodiment, the said reservoir (20) comprises a substantially cylindrical side wall (22), though other shapes may also be suitable. Preferably, the said bottom end (28) is substantially convex or alternatively frusta-conical, in order to minimise sedimentation thereat. The reservoir (20) comprises an internal volume (30) which is typically between 5 and 50 litres, though may be greater than 50 litres or less than 5 litres. Said internal volume (30) may be filled with a suitable sterile biological cell and/or tissue culture medium (50) and/or sterile inoculant (60) and/or sterile air and/or other sterile required additives, as hereinafter described. In the preferred embodiment, the reservoir (20) is substantially non-rigid, being made from materials such as polyethylene or polycarbonate, for example. Optionally, the said reservoir (20) comprises two concentric outer walls (24) to enhance mechanical strength and to minimise risk of contamination of the contents via the reservoir walls. In a different embodiment, the reservoir may be rigid and made from a suitable plastic material. In all embodiments, the reservoir (20) is made from a material or materials that enable the reservoir to be sterilised prior to first use.

The reservoir (20) comprises air inlet means for introducing sterile air in the form of bubbles (70) into said culture medium (50) through a first inlet opening (72) connectable to a suitable air supply (not shown). In the preferred embodiment, said

air inlet means comprises a pipe (74) extending from said inlet opening (72) to a location inside said reservoir (20) at or near said bottom end (28). The pipe (74) thus comprises an air outlet (76) of suitable diameter to produce air bubbles (70) of a required mean diameter. These bubbles not only aerate the medium (50), but also serve to mix the contents of the reservoir. In some cases, particularly relating to plant cells, small bubbles may actually damage the cell walls, and a mean bubble diameter of not less than 4mm substantially overcomes this potential problem. Optionally, said outlet (76) may be restrained in position at said bottom end (28) by means of a tether (not shown) or other means known in the art.

The said reservoir (20) further comprises gas outlet means for removing excess air and/or waste gases from said reservoir (20). These gases collect at the said top end (26) of the said reservoir (20). The said gas outlet means may comprise a pipe (90) having an inlet (96) at or near the said top end (26), said pipe (90) being connectable by known means to a suitable exhaust means (not shown). In a preferred embodiment, the said exhaust means comprises suitable suction means such as a suction pump, for example. The said exhaust means further comprises means for substantially preventing introduction of contaminants into said reservoir via said gas outlet means. In the preferred embodiment, said means for substantially preventing introduction of contaminants comprises a suitable one-way valve.

The said reservoir (20) further comprises additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said reservoir. In the preferred embodiment, said additive inlet means comprises a suitable pipe (80) having an outlet (86) at or near the said top end (26). Said pipe (80) is connectable by known means to a suitable sterilised supply of said inoculant and/or said culture medium and/or said additives. Said additive inlet means further comprises means for substantially preventing introduction of contaminants into said reservoir via said

additive inlet means, and comprises, in the preferred embodiment, a suitable one-way valve (84).

The said reservoir (20) further comprises harvesting means for harvesting a desired first portion of the said medium containing cells and/or tissue, wherein a remaining second portion of said medium containing cells and/or tissue serve as inoculant for a next culture and harvest cycle wherein said culture medium and/or said required additives provided. In the preferred embodiment, said harvesting means comprises a pipe (50) having an inlet (52) in communication with said internal volume (30), and an outlet (56) outside said reservoir (20). Preferably, said inlet (52) is located near the bottom end (28) of the said reservoir (20), so that only the reservoir contents above said inlet (52) are harvested. Thus, at the end of each harvesting cycle, said second portion of medium containing cells and/or tissue automatically remains at the said bottom end (28) of the said reservoir (20), up to a level below the level (51) of the said inlet (52). Alternatively, said inlet (52) may be located at the lowest point in the said reservoir (20), wherein the operator would manually ensure that a suitable portion of medium containing cells and/or tissue would remain in the reservoir (20) after harvesting a desired portion of medium and cells and/or tissue. Said harvest means further comprises means for substantially preventing introduction of contaminants into said reservoir via said harvesting means, and comprises, in the preferred embodiment, a suitable one-way valve (54). Said pipe (50) is of a relatively large diameter, typically 2cm, since the harvested cell and/or tissue flow therethrough is turbid.

In the preferred embodiment, said remaining second portion of said medium containing cells and/or tissue typically comprises between 10% and 20% of the original volume of said culture medium and said inoculant, though said second

portion may be greater than 20% or less than 10% of the said original volume, if required.

Said device (10) optionally further comprises attachment means for attaching same to an overhanging support structure. In the preferred embodiment, said support structure may comprise a bar or rings (not shown), and said attachment means may comprise a hook (25) preferably integrally attached to the said top end (26) of the said reservoir (20).

The present invention also relates to a battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue in cycles, wherein each of a plurality of these devices is structurally and operationally similar to said device (10), hereinbefore defined.

Referring to Figure 2, a battery (500) comprises a plurality of said devices (10) are held on a frame (not shown) by means of said attachment means. A suitable air compressor (100) provides air, sterilised by suitable means at (110) such as suitable filters, to each said device (10) via common piping (174) suitably attached to each corresponding air inlet means. Sterilised air is typically provided continuously during the culturing cycle.

Suction means (200) removes excess air and/or waste gases from each of the said devices (10) via common piping (290) suitably connected to each corresponding gas outlet means. Said suction means is provided with suitable means (210), such as filters, for preventing contaminants from flowing into each said device (10).

In one embodiment, concentrated media in tank (320) is mixed with distilled water from a tank or other suitable supply (330) in a mixing tank (300) and delivered to each said device (10) via a filter (310) and common piping (380) suitably connected to each corresponding additive inlet means. In said mixing tank (300), sterile required additives may be added as well as inoculant prior to delivery to each said device (10). The tank (300) is filled with sufficient concentrated medium to supply all the said devices (10) in the said battery (500) for one cycle. Alternatively, a single pipe or a set of pipes (not shown) connect said mixing tank to a said device (10) or a corresponding set of devices (10), respectively, at a time, wherein a conveyor system transports the device (10) or set of devices (10) to the said single pipe or set of pipes, respectively, or vice versa. After filling the said device (10) or set of devices (10), the conveyor enables a further device (10) or set of devices (10) to be connected to the mixing tank (300) by means of the said single pipe or set of pipes, respectively.

Each device (10) may be harvested by removing said first portion of medium containing cells and/or tissue into corresponding receiving tanks (400) via suitable sterilised connection means. Alternatively, common piping (not shown) may be utilised to connect each harvesting means of each device (10), via suitable sterilised connecting means, to a common receiving tank (not shown). Alternatively, a single pipe or a set of pipes (not shown) may connect said common receiving tank to a said device (10) or a corresponding set of devices (10), respectively, at a time, wherein a conveyor system transports the device (10) or set of devices (10) to the said single pipe or set of pipes, respectively, or vice versa. After harvesting the said device (10) or set of devices (10), the conveyor enables a further device (10) or set of devices (10) to be connected to the said common receiving tank by means of the said single pipe or set of pipes, respectively.

The present invention also relates to a method for culturing and harvesting cells and/or tissue in a multiple-use disposable device comprising the steps of:-

- a) providing said device (10), hereinbefore defined ;
- b) providing sterile air continuously to said reservoir via said air inlet means during each cycle;
- c) providing sterile inoculant and/or sterile said culture medium and/or sterile said additives via said additive inlet means;
- d) optionally illuminating said reservoir with external light means;
- e) allowing said cells and/or tissue to grow in said medium to a desired yield;
- f) continuously allowing excess air and/or waste gases to leave said reservoir via said gas outlet means;
- g) checking for contaminants in said reservoir: if contaminants are found in levels in excess of a threshold, the device and its contents are disposed of; if contaminants are not found, or if the levels thereof are below said threshold, step h) is executed;
- h) harvesting said desired first portion of the said medium containing cells and/or tissue, while leaving said second portion of medium containing cells and/or tissue in said reservoir, wherein said second portion of medium serves as inoculant for a next culture/harvest cycle;

i) providing sterile said culture medium and/or sterile said additives for the next culture/harvest cycle via said additive inlet means;

j) repeating steps b), d), e), f), g), h) and i) a plurality of times until in g) the said contaminants are found in levels in excess of a threshold, whereupon the device and its contents are disposed of.

The present invention also relates to a method for culturing and harvesting cells and/or tissue in a battery of multiple-use disposable device comprising the steps of:-

A) providing a battery of said devices (10), hereinbefore defined ;

B) for each said device, providing steps b) to j) as hereinbefore defined.

Typically, a water distiller supplies distilled water to a tank comprising concentrated media, and diluted media is then pumped to the said device (10) via said additive inlet means. Filters, typically $0.2\mu\text{m}$, are installed in the feed pipes and also just upstream of the said additive inlet means to minimise risk of contamination of the reservoir contents in each device (10). Alternatively or additionally, a one-way valve may be also be used to minimise this risk.

For the first culturing cycle of each device (10), inoculant, typically a sample of the type of cell that it is required to harvest in the said device (10), is pre-mixed with media in a steam sterilised container prior to introducing the media into said device (10), though for subsequent cycles, only media is introduced, as hereinbefore described.

Typically, an air compressor provides substantially sterilised air to each said device (10), via a filter, typically $0.2\mu\text{m}$, wherein particles, humidity and contaminants are

removed. Preferably, another filter just upstream of the said air inlet means further minimises the risk of contamination of the reservoir contents.

For each said device (10), all connections to the reservoir, i.e., to said air inlet means, to said additive inlet means, and preferably also to the gas outlet means and to the harvesting means are sterilised prior to and during connection to peripheral equipment, including, for example, said air supply and said exhaust means. This may be achieved by means of an ultraviolet generation chamber which is provided to enclose each of the connectors and the corresponding peripheral equipment, and irradiating the mating parts of the connectors for a period, typically in the order of minutes, prior to and during connection.

Temperature control for each device (10) is preferably provided by suitable air conditioning means, and optional illumination of the device may be provide by suitable fluorescent light means suitably arranged around the said device (10).

During each culturing cycle of each device (10), the contents of each corresponding reservoir (20) are typically aerated and mixed for 7 to 14 days under controlled temperature and lighting conditions.

At the end of the culturing cycle for each device (10), , the corresponding said harvesting means is typically connected to a presterilised environment by means of suitable connectors which are sterilised prior and during connection, as hereinbefore described. Harvesting is then effected, leaving behind typically 10%-20% of cells and/or tissue to serve as inoculant for the next cycle.

The harvested cells may then be dried, as required.

Although only a few embodiments have been described in detail in the foregoing description, the present invention is not limited thereto and is only defined by the scope of the claims.

CLAIMS

1. A multiple-use disposable device for culturing and harvesting cells and/or tissue in cycles comprising a sterilisable transparent and/or translucent reservoir having a top end and a bottom end, which reservoir may be filled with a suitable sterile biological cell and/or tissue culture medium and/or sterile inoculant and/or sterile air and/or other sterile required additives, said reservoir comprising:-

- (i) air inlet means for introducing sterile air in the form of bubbles into said culture medium through a first inlet opening connectable to a suitable air supply;
- (ii) gas outlet means for removing excess air and/or waste gases from said reservoir connectable to a suitable exhaust means, said exhaust means comprising means for substantially preventing introduction of contaminants into said reservoir via said gas outlet means;
- (iii) additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said reservoir, said additive inlet means comprising means for substantially preventing introduction of contaminants into said reservoir via said additive inlet means; and
- (iv) harvesting means for harvesting a desired first portion of the said medium containing cells and/or tissue, wherein a remaining second portion of said medium containing cells and/or tissue serve as inoculant for a next culture and harvest cycle wherein said culture medium and/or said required additives provided, said harvest means comprising means for substantially preventing introduction of contaminants into said reservoir via said harvesting means;

wherein said device may be utilised a plurality of times for culturing and harvesting said cells and/or tissue in cycles, wherein inoculant is externally provided for a first cycle thereof, and wherein for each subsequent cycle, said second portion of medium with cell/tissue remaining after a preceding harvest serves as inoculant for the next

cycle, and wherein medium and air and any other required additives are provided in suitable quantities during each cycle to enable culture of said cells and/or tissue from said inoculant, and wherein said device may be disposed of when contaminated.

2. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said reservoir is substantially cylindrical.
3. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said reservoir is non-rigid.
4. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in claim 3, wherein said reservoir is made from polyethylene or from polycarbonate.
5. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said reservoir comprises two concentric outer walls.
6. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any one of claims 1 or 2, wherein said reservoir is substantially rigid.
7. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said air inlet means comprises a pipe extending from said inlet opening to a location inside said reservoir at or near the said bottom end thereof.

8. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein at least a majority of said air bubbles comprise a mean diameter of not less than 4mm.
9. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said means for substantially preventing introduction of contaminants into said reservoir via said gas outlet means comprises a suitable one-way valve.
10. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said means for substantially preventing introduction of contaminants into said reservoir via said additive inlet means comprises a suitable one-way valve.
11. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said means for substantially preventing introduction of contaminants into said reservoir via said harvesting means comprises a suitable one-way valve.
12. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said exhaust means comprises a suitable suction generation means.
13. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said harvesting means is located at said bottom end of said reservoir.

14. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any one of claims 1 to 12, wherein said harvesting means is located near the said bottom end of said reservoir, such that at the end of each harvesting cycle said remaining second portion of said medium containing cells and/or tissue automatically remains at the said bottom end of said reservoir up to a level below the level of said harvesting means.
15. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said remaining second portion of said medium containing cells and/or tissue comprises between 10% and 20% of the original volume of said culture medium and said inoculant.
16. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said bottom end is substantially convex.
17. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any one of claims 1 to 15, wherein said bottom end is substantially frustaconical.
18. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said reservoir comprises an internal volume of between 5 and 50 litres.
19. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein said device further comprises suitable attachment means for attaching same to a suitable support structure.

20. A multiple-use disposable device for culturing and harvesting cells and/or tissue as claimed in claim 19, wherein said attachment means comprises a hook preferably integral attached to said top end of said reservoir.

21. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue in cycles, each said device comprising a sterilisable transparent/translucent reservoir having a top end and a bottom end, which reservoir may be filled with a suitable sterile biological cell/tissue culture medium and/or sterile inoculant and/or sterile air and/or other sterile required additives, said reservoir comprising:-

- (i) air inlet means for introducing sterile air in the form of bubbles into said culture medium through a first inlet opening connectable to a suitable air supply;
- (ii) gas outlet means for removing excess air and/or waste gases from said reservoir connectable to a suitable exhaust means, said exhaust means comprising means for substantially preventing introduction of contaminants into said reservoir via said gas outlet means;
- (iii) additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said reservoir, said additive inlet means comprising means for substantially preventing introduction of contaminants into said reservoir via said additive inlet means; and
- (iv) harvesting means for harvesting a desired first portion of the said medium containing cells and/or tissue, wherein a remaining second portion of said medium containing cells and/or tissue serve as inoculant for a next culture and harvest cycle wherein said culture medium and/or said required additives provided, said harvest means comprising means for substantially preventing introduction of contaminants into said reservoir via said harvesting means;

wherein each said device may be utilised a plurality of times for culturing and harvesting said cells and/or tissue in cycles, wherein inoculant is externally provided for a first cycle thereof, and wherein for each subsequent cycle, said second portion of medium with cell/tissue remaining after a preceding harvest serves as inoculant for the next cycle, and wherein medium and air and any other required additives are provided in suitable quantities during each cycle to enable culture of said cells and/or tissue from said inoculant, and wherein each said device may be disposed of when contaminated.

22. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in claim 21, wherein for each said device said reservoir is substantially cylindrical.
23. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said reservoir is non-rigid.
24. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in claim 23, wherein for each said device said reservoir is made from polyethylene or from polycarbonate.
25. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said reservoir comprises two concentric outer walls.
26. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any one of claims 21 or 22, wherein for each said device said reservoir is substantially rigid.

27. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said air inlet means comprises a pipe extending from said inlet opening to a location inside said reservoir at or near the said bottom end thereof.
28. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device at least a majority of said air bubbles comprise a mean diameter of not less than 4mm.
29. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said means for substantially preventing introduction of contaminants into said reservoir via said gas outlet means comprises a suitable one-way valve.
30. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said means for substantially preventing introduction of contaminants into said reservoir via said additive inlet means comprises a suitable one-way valve.
31. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said means for substantially preventing introduction of contaminants into said reservoir via said harvesting means comprises a suitable one-way valve.
32. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said exhaust means comprises a suitable suction generation means.

33. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said harvesting means is located at said bottom end of said reservoir.
34. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any one of claims 21 to 32, wherein for each said device said harvesting means is located near the said bottom end of said reservoir, such that at the end of each harvesting cycle said remaining second portion of said medium containing cells and/or tissue automatically remains at the said bottom end of said reservoir up to a level below the level of said harvesting means.
35. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said remaining second portion of said medium containing cells and/or tissue comprises between 10% and 20% of the original volume of said culture medium and said inoculant.
36. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said bottom end is substantially convex.
37. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any one of claims 1 to 15, wherein for each said device said bottom end is substantially frusta-conical.

38. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said reservoir comprises an internal volume of between 5 and 50 litres.

39. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in any preceding claim, wherein for each said device said device further comprises suitable attachment means for attaching same to a suitable support structure.

40. A battery of multiple-use disposable devices for culturing and harvesting cells and/or tissue as claimed in claim 39, wherein for each said device said attachment means comprises a hook preferably integral attached to said top end of said reservoir.

41. A method for culturing and harvesting cells and/or tissue in a multiple-use disposable device comprising the steps of :-

a) providing said device which comprises a transparent and/or translucent reservoir having a top end and a bottom end, which reservoir may be filled with a suitable sterile biological cell and/or tissue culture medium and/or sterile inoculant and/or sterile air and/or other sterile required additives, said reservoir comprising:-

- (i) air inlet means for introducing sterile air in the form of bubbles into said culture medium through a first inlet opening connectable to a suitable air supply;
- (ii) gas outlet means for removing excess air and/or waste gases from said reservoir connectable to a suitable exhaust means, said exhaust means comprising means for substantially preventing introduction of contaminants into said reservoir via said gas outlet means;

- (iii) additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said reservoir, said additive inlet means comprising means for substantially preventing introduction of contaminants into said reservoir via said additive inlet means; and
 - (iv) harvesting means for harvesting a desired first portion of the said medium containing cells and/or tissue, wherein a remaining second portion of said medium containing cells and/or tissue serve as inoculant for a next culture and harvest cycle wherein said culture medium and/or said required additives provided, said harvest means comprising means for substantially preventing introduction of contaminants into said reservoir via said harvesting means;
- b) providing sterile air continuously to said reservoir via said air inlet means during each cycle;
 - c) providing sterile inoculant and/or sterile said culture medium and/or sterile said additives via said additive inlet means;
 - d) optionally illuminating said reservoir with external light means;
 - e) allowing said cells and/or tissue to grow in said medium to a desired yield;
 - f) continuously allowing excess air and/or waste gases to leave said reservoir via said gas outlet means;
 - g) checking for contaminants in said reservoir: if contaminants are found in levels in excess of a threshold, the device and its contents are disposed of; if

contaminants are not found, or if the levels thereof are below said threshold, step h) is executed;

- h) harvesting said desired first portion of the said medium containing cells and/or tissue, while leaving said second portion of medium containing cells and/or tissue in said reservoir, wherein said second portion of medium serves as inoculant for a next culture/harvest cycle;
- i) providing sterile said culture medium and/or sterile said additives for the next culture/harvest cycle via said additive inlet means;
- j) repeating steps b), d), e), f), g), h) and i) a plurality of times until in g) the said contaminants are found in levels in excess of a threshold, whereupon the device and its contents are disposed of.

42. A method for culturing and harvesting cells and/or tissue in a battery of multiple-use disposable devices comprising the steps of :-

- a) providing a battery of said devices, each said device of which comprises a transparent and/or translucent reservoir having a top end and a bottom end, which reservoir may be filled with a suitable sterile biological cell and/or tissue culture medium and/or sterile inoculant and/or sterile air and/or other sterile required additives, said reservoir comprising:-
 - (i) air inlet means for introducing sterile air in the form of bubbles into said culture medium through a first inlet opening connectable to a suitable air supply;

- (ii) gas outlet means for removing excess air and/or waste gases from said reservoir connectable to a suitable exhaust means, said exhaust means comprising means for substantially preventing introduction of contaminants into said reservoir via said gas outlet means;
- (iii) additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said reservoir, said additive inlet means comprising means for substantially preventing introduction of contaminants into said reservoir via said additive inlet means; and
- (iv) harvesting means for harvesting a desired first portion of the said medium containing cells and/or tissue, wherein a remaining second portion of said medium containing cells and/or tissue serve as inoculant for a next culture and harvest cycle wherein said culture medium and/or said required additives provided, said harvest means comprising means for substantially preventing introduction of contaminants into said reservoir via said harvesting means;

b) and for each said device:-

- I) providing sterile air continuously to said reservoir via said air inlet means during each cycle;
- II) providing sterile inoculant and/or sterile said culture medium and/or sterile said additives via said additive inlet means;
- III) optionally illuminating said reservoir with external light means;
- IV) allowing said cells and/or tissue to grow in said medium to a desired yield;

- V) continuously allowing excess air and/or waste gases to leave said reservoir via said gas outlet means;
- VI) checking for contaminants in said reservoir: if contaminants are found in levels in excess of a threshold, the device and its contents are disposed of; if contaminants are not found, or if the levels thereof are below said threshold, step VII) is executed;
- VII) harvesting said desired first portion of the said medium containing cells and/or tissue, while leaving said second portion of medium containing cells and/or tissue in said reservoir, wherein said second portion of medium serves as inoculant for a next culture/harvest cycle;
- VIII) providing sterile said culture medium and/or sterile said additives for the next culture/harvest cycle via said additive inlet means;
- IX) repeating steps I), III), IV), V), VI), VII) and VIII) a plurality of times until in VI) the said contaminants are found in levels in excess of a threshold, whereupon the said device and its contents are disposed of.


A. E. MULFORD
ATTORNEYS FOR APPLICANTS

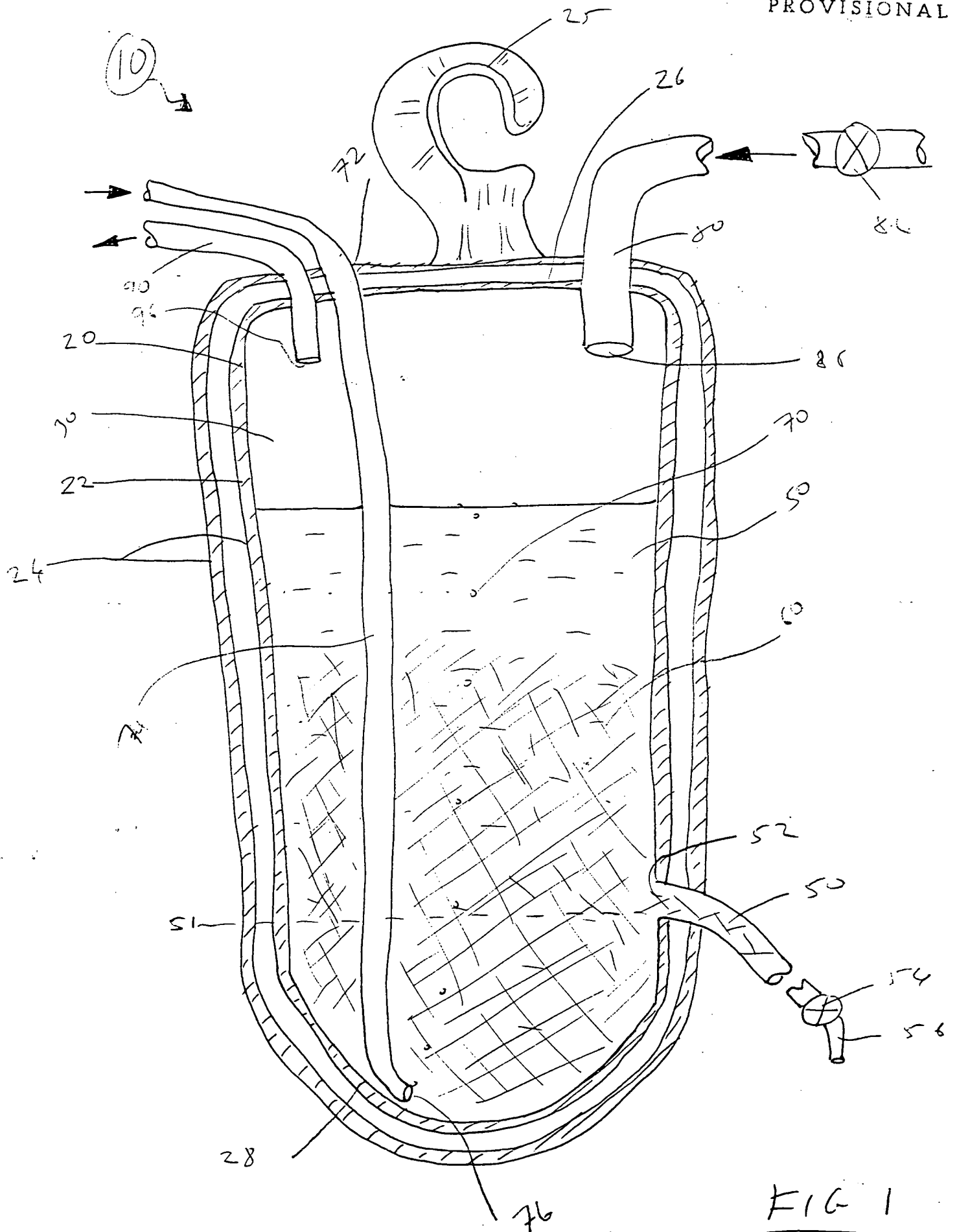


FIG 1

